Osteoporosis, which increases the risk of bone fractures, is becoming one of the major public health concerns in the world, mainly because of the rapid aging of the population. Although more than 50% of the variance in peak bone mass is attributable to genetic factors (1), there exist manageable environmental factors that can modify bone growth, such as nutritional intake (2) and physical activity (3). It is estimated that a 10% increase in peak bone mass could delay the development of osteoporosis by 13 y (4) and reduce the risk of osteoporotic fractures after menopause by 50% (5). Thus, maximizing the peak bone mass is considered to be an important approach to preventing fractures in later years. Given that, searching for factors leading to a higher peak bone mass during childhood seems important.

Associations between bone growth and calcium intake have been studied intensively. There are a number of studies that have shown the effectiveness of calcium on bone mineralization (6, 7); however, several studies did not demonstrate any such role (8, 9). There may be several reasons for these conflicting results. Bone growth is derived not only from calcium intake but also from other nutrients such as protein and several kinds of vitamins (2). It is necessary to evaluate not only the nutrients but also a subject’s physical activity level, as a combined effect of physical activity level and nutrient intake has been suggested (10). Furthermore, menstrual status affects the nutrient function (11), and thus it is necessary to take the influence of nourishment, physical activity level, and sex hormone status into account overall to search for the factors affecting bone growth. However, there is little research concerning Japanese children before reaching the maximal bone mass (12), and the promotion factors for bone growth have not been determined.

The time for the acquisition of bone mass is limited, and most of bone sites have reached their peaks by the

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**Search for Promotion Factors of Ultrasound Bone Measurement in Japanese Males and Pre/Post-Menarcheal Females Aged 8–14 Years**

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(Received February 20, 2012)

**Summary** There is little evidence regarding the associations between bone growth and environmental factors among growing children, especially in Asians. The aim of this cross-sectional study was to search for the promotion factors of bone growth in Japanese children during growth. The study subjects were male (n=333) and pre/post-menarcheal female (n=179/n=68) school children aged 8–14 y. Bone status at the calcaneus was evaluated by quantitative ultrasound (Benus III), and the bone area ratio (BAR) was used as an evaluation index. Dietary intakes were assessed via brief self-administered diet history questionnaires. The participants were asked to record all of their activities for 3 d (2 weekdays and 1 holiday). They were also required to provide the most recent anthropometric measurement records at their schools and answer questions about the frequency of fractures and, for females, the length of time since menarche. Multiple regression analysis with dummy variables demonstrated that age, magnesium (more than the RDA), vitamin B1 (more than the RDA), mean physical activity intensity per day (more than 1.7 METs), vitamin C (more than the RDA) and calcium (more than the RDA) were significantly positive influential factors of BAR for males. For premenarcheal females, age, vitamin A (more than the RDA), BMI, and mean physical activity intensity per day (more than 1.7 METs) were significantly positive influential factors of BAR, and for postmenarcheal females, only BMI and age were significantly positive influential factors of BAR. The results suggest that several manageable factors correlate with the bone mass, and the associations differ depending on gender and menarcheal status.

**Key Words** bone area ratio, growing children, menarche, nutrients, physical activity

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end of the second decade of life (2). The peak accumulation of bone mass measured at the calcaneus with quantitative ultrasound (QUS) was reported to occur between 11 and 13 y of age in Japanese males and females (1, 3). Other studies have reported similar results in Western adolescents (14, 15). Therefore, we performed multiple regression analysis to investigate the association between the bone growth at the calcaneus with QUS and the factors such as major nutrients, physical activity level, anthropometry, and length of time since menarche in 8- to 14-y-old Japanese children, as this is the age during which their bones are growing.

We proposed the following three hypotheses: 1) A threshold of nutrient intake and physical activity level may exist for bone growth, and the relationship between bone growth and nutrient intake or physical activity level may not always be linear. 2) In growing children, the factor that promotes bone growth may have a sex difference. 3) In growing females, the factor that promotes bone growth may have a difference by menarchal status. Therefore, we used not only the continuous variables but also the categorized variables as independent variables for multiple regression analysis. All analyses were performed separately by gender, and female data were analyzed separately according to whether menarche had occurred.

The aim of this study was to test our three hypotheses and thereby to determine the factors that contribute to bone growth in Japanese children during growth.

SUBJECTS AND METHODS

Subjects. The 444 pupils from 3rd to 5th grade (aged 8–11 y) attending private and public elementary schools and the 219 students from 1 to 2nd grade (aged 12–14 y) attending private junior high schools in the Kinki region were told the object of the research, in accordance with the Helsinki Declaration of June 2009. Informed consent for study participation was obtained from each of the participants and from their parent or guardian. This study was conducted with the approval of the Kinki University Faculty of Agriculture life ethics committee. A total of 406 elementary school pupils and 190 junior high school students participated in this study. Excluded from the present analysis were 16 subjects who reported extremely low or high energy intake (that is, <0.5 times the estimated energy requirement value for the lowest physical activity category or >1.5 times that for the highest physical activity category according to the Dietary Reference Intakes for Japanese issued in 2010). The exclusion criteria in the study of Murakami et al. (16) were applied in the present study. Of the total 580 subjects for the present analysis, 100 were in the 3rd grade, 222 were in the 4th grade, 78 were in the 5th grade, 92 were in the 7th grade, and 88 were in the 8th grade. For males, the age breakdown was as follows: 8 y of age (n=49), 9 y (n=111), 10 y (n=68), 11 y (n=7), 12 y (n=38), 13 y (n=48), and 14 y (n=12). For females, the age breakdown was as follows: 8 y of age (n=28), 9 y (n=88), 10 y (n=42), 11 y (n=7), 12 y (n=37), 13 y (n=35), and 14 y (n=10). No 6th grade subjects were included, because there was no opportunity to include examinations in the school curriculum for that age group. In addition, the administrators at one elementary school hoped for the participation of 4th graders only. Consequently, there was a small number of participants who were 11 y old, and there were more participants who were 9 y old than there were participants of other ages.

All investigations were conducted from June through July of 2009.

Anthropometric measurement. Data for body weight (kg) to the nearest 0.1 kg while wearing light clothes and standing height (cm) to the nearest 0.1 cm were obtained from the most recent anthropometric measurement records at subjects’ schools.

Bone measurement. The bone status of the right calcaneus was evaluated with QUS using Benus III (Ishikawa Seisakusho, Ltd., Japan). Benus III is equipped with two kinds of L-shaped spacer to refine the position of ultrasound waves. The kids-spacer (10-mm thick) was used for feet ≤18 cm in length, and the M-spacer (5-mm thick) was used for feet >18 cm and <23 cm in length. These spacers were not normally used for feet equal to or more than 23 cm long, except in children with flat feet. In addition, Benus III shows a “Results judgment of the ultrasound reflection distance,” which is an indication of whether the position of the ultrasound wave is proper. We measured calcaneal bone according to the Benus III instruction manual.

The definition of “bone growth” in this study was the bone status at the calcaneus evaluated by Benus III, and the bone area ratio (BAR) was used as an evaluation index. BAR refers to the percentage of the area that bone trabeculae (ossein) occupy in the sectional area, and has been shown to have a correlation (r=0.83, p<0.01) with bone mineral density by dual-energy X-ray absorptiometry (DXA) measurement at the calcaneus (17, 18). The coefficient of variation of the densitometer was reported to be 0.8% for SOS and 1.6% for BAR at the calcaneus (17).

Benus III measures the ultrasonic wave penetration time and the width of the calcaneus bone. BAR was calculated using the following equation:

\[ BAR = \frac{Eu^2 - (a/b)^2}{a(T_2 - T_1)/(1/Va - 1/Vw)} \]

where \( b \) = length of cancellous bone, \( T_1 \) = conduction time only of marrow, \( T_2 \) = conduction time of cancellous bone, \( Va \) = velocity of conduction in solid tissue, \( Vw \) = velocity of conduction in liquid inside, and \( Eu \) (bone trabeculae line density) = length of ossein (a)/length of cancellous bone (b) (17, 19).

Assessment of nutrient intake and general questionnaire. Nutrient intake was assessed with the following self-administered diet history questionnaires. Sasaki modified a brief self-administered diet history questionnaire (BDHQ), previously validated for adults (20), to assess the habitual diet for children and adolescents. Okuda et al. demonstrated the validity of the modified BDHQ for junior high school students (BDHQ-15y) with biomarkers (21). As another modified BDHQ for elementary
school children, the BDHQ-10y was reported to show improved accuracy of dietary estimation when children answered with the help of their parent or guardian (21). In the present study nutrient intake was assessed with the BDHQ-10y for elementary school pupils aged 8–11 y and with the BDHQ-15y for junior high school students aged 12–14 y. The BDHQ-10y and BDHQ-15y inquired about diet history during the preceding month with regard to 54 and 67 food items, respectively. Intake of 99 nutrients can be calculated using each questionnaire (21). The questionnaires were distributed to the children at school with a request for the parent or guardian to help children provide the answers. The participants completed the questionnaires with their parent/guardian at home and returned them to their classroom teachers. A general questionnaire about frequency of fractures, medicines taken for bone growth, sleeping time, and, for the girls, the length of time since menarche was also distributed, and the participants were required to answer at school.

Assessment of mean physical activity intensity per day. The participants were asked to record, with the help of their parent/guardian, all of their activities at home for 3 d (2 weekdays and 1 holiday) so we could estimate their mean physical activity intensity per day. The participants were exempted from recording the common activities during school hours, to reduce the work. The common activities during school hours were estimated from the timetables of each school. The physical activity intensity of each day was evaluated as the metabolic equivalent (MET) intensity according to the physical activity level codes as METs (22).

The mean physical activity intensity level for a single day was estimated using the following equation:

Mean physical activity intensity level for a single day (METs) = Σ [Intensity of each activity (METs) × Time of each activity (min)] ÷ 1.440 (min)

The mean physical activity intensity level per day was then estimated using the following equation:

Mean physical activity intensity level per day (METs) = [(Mean METs at a weekday + Mean METs at another weekday) ÷ 2 × 5 + Mean METs at a holiday] ÷ 6

Statistical analysis. All statistical analyses were performed with SPSS version 18.0 for Windows. All analyses were performed separately by gender. In addition, female data were analyzed separately according to whether menarche had occurred. Descriptive results were expressed as means and standard deviations. Multiple regression analysis was used to find significant associations between BAR at the calcaneus as an outcome variable and environmental factors such as nutrient intake and physical activity intensity as independent variables. The independent variables selected for inclusion in the model were determined by both those suggested in a previous study (23) and potential predictors for bone mass (24). The independent continuous variables were preferentially entered in the model. That is, age, height, weight, body mass index (BMI), mean physical activity intensity per day (METs), sleeping time, frequency of fractures, and length of time since menarche (only female) as non-nutrient continuous variables, and the intake of protein, lipids, carbohydrates, potassium, calcium, magnesium, phosphorus, iron, zinc, vitamin A as retinol, vitamin B1, vitamin B2, vitamin B6, vitamin B12, niacin, vitamin C, vitamin D, vitamin E, vitamin K, folic acid, dietary fiber, and salt as major nutrient continuous variables were entered into a multiple regression model with a backward elimination procedure. Only variables that approached the errors of a p value less than 5% were retained in the model. The continuous variables that had been removed from the model were categorized with dummy variables, and we then tried to reinsert them in the model. For instance, the intake of nutrients other than dietary fiber were categorized into two groups according to each recommended dietary allowance (RDA) [or estimated average requirement (EAR) or adequate intake (AI) or tentative dietary goal for preventing life-style related disease (DG)] in Dietary Reference Intakes for Japanese issued in 2010, and we then tried to reinsert them in the model as dummy variables as follows: 0 = less than the RDA (or EAR or AI or DG); 1 = equal to or more than the RDA (or EAR or AI or DG). Dietary fiber intake was categorized into two groups according to each average value, because there is no dietary reference intake for Japanese aged 0–17 y. As for the mean physical activity intensity per day, the continuous variables were categorized as follows: 0 = less than 1.4 METs (or 1.5 or 1.6 or 1.7 or 1.8 METs); 1 = equal to or more than 1.4 METs (or 1.5 or 1.6 or 1.7 or 1.8 METs). When the p value was less than 5% error, the dummy variables were retained in the model. In addition, the multicollinearity of inde-
Table 1. Basic characteristics of subjects and ultrasound bone measurements.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Males (n=333)</th>
<th>Females (n=247)</th>
<th>Premenarche (n=179)</th>
<th>Postmenarche (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nutrient</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>10.2±1.8</td>
<td>10.3±1.8</td>
<td>9.5±1.2***</td>
<td>12.6±0.8</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>141.0±12.1</td>
<td>141.2±11.6</td>
<td>136.2±9.6***</td>
<td>154.4±5.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>35.6±10.9</td>
<td>34.7±9.0</td>
<td>31.0±6.9***</td>
<td>44.5±6.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.5±2.9</td>
<td>17.1±2.4</td>
<td>16.6±2.3***</td>
<td>18.6±2.1</td>
</tr>
<tr>
<td>Mean PA intensity (METs)¹</td>
<td>1.6±0.3***</td>
<td>1.5±0.2</td>
<td>1.6±0.2***</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>Sleeping time (min)</td>
<td>519.0±67.8</td>
<td>513.1±57.7</td>
<td>532.5±48.1***</td>
<td>462.2±49.7</td>
</tr>
<tr>
<td>SOS at calcaneus (m/s)</td>
<td>1.613±25.6</td>
<td>1.612±30.0</td>
<td>1.602±24.1***</td>
<td>1.636±30.5</td>
</tr>
<tr>
<td>BAR at calcaneus (%)</td>
<td>30.4±3.1*</td>
<td>31.1±4.1</td>
<td>30.1±3.3***</td>
<td>34.0±4.6</td>
</tr>
<tr>
<td>Length of time since menarche (mo)</td>
<td>17.2±12.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Mean PA intensity (METs) was estimated from records of activity records for 3 d, used to determine the mean physical activity intensity levels per day.

Table 2. Intake of major nutrients per day assessed by brief self-administered diet history questionnaires.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Males (n=333)</th>
<th>Females (n=247)</th>
<th>Premenarche (n=179)</th>
<th>Postmenarche (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nutrient</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>81.1±22.0***</td>
<td>69.5±18.6</td>
<td>69.2±17.0</td>
<td>70.4±22.5</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>76.5±20.4***</td>
<td>68.0±18.7</td>
<td>66.7±16.2</td>
<td>71.4±23.7</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>308.0±87.3***</td>
<td>257.8±71.2</td>
<td>261.5±68.0</td>
<td>248.2±78.6</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>13.4±4.2***</td>
<td>12.2±3.8</td>
<td>12.6±3.6*</td>
<td>11.0±4.1</td>
</tr>
<tr>
<td>Vitamin A (µgRE)</td>
<td>837.9±410.1**</td>
<td>727.5±206.2</td>
<td>729.1±228.5</td>
<td>723.3±331.6</td>
</tr>
<tr>
<td>Vitamin D (µg)</td>
<td>14.2±7.5**</td>
<td>12.0±6.5</td>
<td>11.7±5.8</td>
<td>12.8±8.0</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>9.6±2.7**</td>
<td>8.8±2.5</td>
<td>8.9±2.4</td>
<td>8.7±2.8</td>
</tr>
<tr>
<td>Vitamin K (µg)</td>
<td>300.5±136.6*</td>
<td>277.2±128.9</td>
<td>283.4±116.2</td>
<td>260.4±157.3</td>
</tr>
<tr>
<td>Vitamin B₁ (mg)</td>
<td>1.0±0.3***</td>
<td>0.8±0.2</td>
<td>0.8±0.2</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>Vitamin B₂ (mg)</td>
<td>1.8±0.5**</td>
<td>1.5±0.4</td>
<td>1.5±0.3</td>
<td>1.6±0.5</td>
</tr>
<tr>
<td>Niacin (mgNE)</td>
<td>16.7±5.3***</td>
<td>14.4±4.5</td>
<td>14.5±4.4</td>
<td>14.1±4.9</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>1.4±0.4***</td>
<td>1.2±0.3</td>
<td>1.3±0.3</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>Vitamin B₁₂ (mg)</td>
<td>9.4±4.4**</td>
<td>8.0±3.9</td>
<td>7.9±3.7</td>
<td>8.4±4.4</td>
</tr>
<tr>
<td>Folic acid (µg)</td>
<td>421.3±120.0***</td>
<td>380.1±110.3</td>
<td>393.4±99.4</td>
<td>345.2±129.5</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>162.6±60.9**</td>
<td>145.6±51.7</td>
<td>154.3±49.3***</td>
<td>122.7±51.2</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>3,053.7±862.5***</td>
<td>2,658.3±723.4</td>
<td>2,710.3±646.7</td>
<td>2,521.5±885.5</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>796.7±307.5***</td>
<td>675.3±283.8</td>
<td>643.5±182.8***</td>
<td>759.1±331.4</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>280.2±76.7****</td>
<td>243.8±63.8</td>
<td>244.3±56.5</td>
<td>242.3±80.2</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>1,308.0±378.9***</td>
<td>1,112.7±304.0</td>
<td>1,090.9±256.2</td>
<td>1,169.9±400.4</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>8.9±2.3***</td>
<td>7.9±2.1</td>
<td>8.0±1.9</td>
<td>7.5±2.5</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>9.7±2.6***</td>
<td>8.3±2.2</td>
<td>8.2±2.0</td>
<td>8.4±2.5</td>
</tr>
<tr>
<td>Salt (g)</td>
<td>11.6±2.6***</td>
<td>10.2±2.4</td>
<td>10.5±2.4</td>
<td>9.5±2.3</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001: Males total vs. Females total (t-test).

RESULTS

The data for 333 males and 247 females were analyzed separately according to sex in this study. The data for females separated by menarche status were also

...dependent variables in the multiple regression model was judged by a variance inflation factor (VIF). Energy and nutrient intake were not entered in the same model, and height, weight, and BMI were not entered in the same model either because of their high degree of collinearity.
The mean and standard deviation for BAR at calcaneus according to gender and age is shown in Fig. 1. The mean of BAR at age 9 was significantly higher than that at age 8 in both genders. In addition, the mean of BAR at age 13 for females was significantly higher than that at age 12. As for the comparison between males and females at the same age, the mean of BAR was significantly higher in females than in males. The mean of BAR at age 8 in both genders, and the mean of BAR at age 13 for females was significantly higher than that at age 12.

Table 1 shows the mean and standard deviation (SD) for numerical nondietary variables. Among the variables examined, mean physical activity intensity per day in males (30.4 METs) was significantly higher than that in total females (27.7 METs, p<0.001), while the mean of BAR in total females (31.1 ± 4.1%) was significantly higher than that in males (28.3 ± 3.8%, p<0.05). The means of all the variables in premenarcheal females were significantly different (p<0.001) from those in postmenarcheal females. The mean length of time since menarche was 17.2 mo in postmenarcheal females.

Table 2 shows the mean ± SD of the intake of major nutrients per day assessed by brief self-administered diet history questionnaires. All the mean nutrient intakes in males were significantly higher than those in total females. Among the females, the mean intakes of dietary fiber (p<0.05) and vitamin C (p<0.001) in premenarcheal females were significantly higher, while the intake of calcium (p<0.01) in premenarcheal females was significantly lower than those in postmenarcheal females.

Table 3 shows the results of multiple regression analysis for the BAR prediction. The model for males demonstrated that age, magnesium (more than the RDA), vitamin B1 (more than the RDA), mean physical activity intensity per day (more than 1.7 METs), vitamin C (more than the RDA), and calcium (more than the RDA) were significantly positive influential factors of BAR, and the adjusted coefficient of determination (adjusted $R^2$) was 0.234 in the model for males. Judging from the standardized partial regression coefficient ($\beta=0.196, p=0.001$), intake of magnesium (more than the RDA) was the most influential factor for BAR in males except for age, which is a non-manageable factor. On the other hand, the model for total females demonstrated that age, BMI, length of time since menarche, and mean physical activity intensity per day were significantly positive influential factors of BAR (adjusted $R^2=0.288$), and BMI except for age was the most important factor judging from $\beta$ value ($\beta=0.184$). Although several dietary variables were observed in the model for males as explanatory variables for BAR, there was no dietary variable correlated with BAR in the model for total females. When female subjects were divided by the experience of menarche, the model for premenarcheal females indicated age, vitamin A (more than the RDA), BMI, and mean physical activity intensity per day (more than 1.7 METs) were significantly positive influential factors of BAR.
factors of BAR (adjusted $R^2=0.159$), and the model for postmenarcheal females demonstrated that only BMI and age were significantly positive influential factors (adjusted $R^2=0.149$). We concluded that there was no multicollinearity in any of the multiple regression models, since the VIFs of all independent variables were less than two.

**DISCUSSION**

To our knowledge, this is the first report to analyze the relationship between bone status and environmental factors such as the intake of nutrients and mean physical activity intensity per day by multiple regression analysis in which the independent variables categorized with dummy variables were entered in the model in place of the continuous variables that were dropped from the model. That is, the continuous variables that had been dropped by the multiple regression analysis with the backward elimination procedure were categorized with dummy variables, and we then tried to re-enter them in the model. Nakagi et al. (12) reported that there was no significant correlation between bone mineral density and any nutritional element was obtained by BDHQ-10y in Japanese juveniles. They analyzed the associations by a multiple regression model using only continuous variables. In our study, the independent continuous variables such as magnesium, vitamin B1, vitamin C, calcium, and mean physical activity intensity per day were dropped from the model for total males, and vitamin A and mean physical activity intensity per day were dropped from the model for premenarcheal females. However, their categorized variables were retained in each model as significant outcome variables. Accordingly, the influence on bone growth of these factors may be decided based on whether those nutrient intakes exceed the respective RDAs or the mean physical activity intensity per day exceeds 1.7 METs. The findings indicate that there is an inflection point for each nutrient and mean physical activity intensity for bone growth, and the beneficial effects of them on bone growth may not be continued linearly after exceeding their inflection points. Regarding information about the calcium balance threshold, Matkovic and Heaney (25) reported that the threshold values at which balance no longer rises with intake exceeded the United States RDA for calcium for the groups aged 0–30 y. It has been observed that only subjects with low daily calcium intake can receive a benefit from increased calcium intake (26). However, the threshold for optimal bone growth was not known, and it may vary with other conditions. Our data suggested that the threshold of calcium intake for bone acquisition was around the Japanese RDA of calcium among Japanese males aged 8–14 y. In addition to calcium, magnesium (more than the RDA), vitamin B1 (more than the RDA), and vitamin C (more than the RDA) for males, and vitamin A (more than the RDA) for premenarcheal females were positively associated with bone growth. Accordingly, dietary intervention to prevent osteoporosis seems to be more effective for Japanese children in whom the nutrient intakes are less than the RDAs.

Judging from $\beta$ values, age was the most influential positive factor for males and total females and was linearly associated with BAR. The results suggested that bone mass increased according to age gain in the participants ranging in age from 8 to 14 y, regardless of the sex. Hirota et al. showed a similar tendency in Japanese boys and girls ranging in age from 10 to 15 y by QUS values (13).

With regard to the nutrients other than calcium, magnesium intake (more than the RDA) was the second-most influential positive factor for BAR in males. Dimai et al. (27) have shown that daily oral magnesium supplementation suppresses bone turnover in young adult males. High magnesium intakes have been associated with higher bone mineral content in western elderly men and women (28). These clinical data support that magnesium may be a positive influential factor for bone mass.

Vitamin B1 intake (more than the RDA) was the third-most influential positive factor for BAR in males. Regarding the relationship between vitamin B1 intake and bone growth, no report has been found. However, there are some reports (29, 30) that show a positive association between energy intake and bone mass. Though energy intake was excluded from the independent variables to avoid collinearity in our study. Vitamin B1 acts as a cofactor for the metabolism of carbohydrates, helping to turn starch and sugar into the energy our bodies need. These activities imply an indirect beneficial effect on bone growth.

Vitamin C intake (more than the RDA) was the fifth-most influential positive factor for BAR in males. However, studies of the association between vitamin C intake and bone status have had conflicting results, probably because there have been many studies of postmenopausal women who had a high prevalence of vitamin C supplement use (for example, in one study 28% of the subjects were regular daily users of vitamin C supplements) (31). In the present study we examined only dietary sources of vitamin C and did not consider supplemental intake, and thus there may be errors in our estimation of a subject’s total vitamin C intake. However, only about 4% of Japanese children aged 7 to 14 y take vitamin C supplements (including vitamin C fortified food) according to the national health and nutrition survey in Japan, 2008. Although the impact of the error may be small, the effect on bone growth of dietary assessment including supplemental intake should be clarified.

Vitamin A intake (more than the RDA) was the second-most influential positive factor for BAR in premenarcheal females. Some epidemiological studies have reported that excessive intake of vitamin A was associated with a low bone mineral density (32, 33) and a risk of fractures (32, 34), while other studies have demonstrated no effects of vitamin A on bone mineral density or fracture risk (35, 36). Thus, many previous reports do not support our result, even though no one exceeded the tolerable upper intake level for vitamin A among the
premenarcheal females in our study. The effects of vitamin A on bone may vary with the age and sex, because an association was observed only in premenarcheal females among our study participants. In most of the previous reports, adults or elderly females were studied. In our data analysis the intake of vitamin A was calculated as a retinol equivalent; however, in previous studies, only retinol but not its equivalent intake has been associated with negative effects on bone health (34). There is a need to analyze the association between bone growth and vitamin A intake that distinguishes between preformed retinol and provitamin A.

Although there is consensus that vitamin D insufficiency or deficiency during childhood is a risk factor for fracture in later life (37, 38), we could not demonstrate the truth or falseness of this belief. Vitamin D is obtained either from the diet or by synthesis in the skin under the action of sunlight (39). We evaluated vitamin D status based only on diet; however, sunlight exposure may be a more important factor in determining vitamin D status. The effect on bone growth of sunlight exposure should be clarified.

Regarding the effects of physical activity on BAR, mean physical activity intensity per day was a positive influential factor for total females, and categorized mean physical activity intensity per day (more than 1.7 METs) was a positive influential factor for males and premenarcheal females; however, a significant association was not observed for postmenarcheal females in our study. Many studies have found a significant relationship between physical activities and bone growth for prepubertal boys (40), adolescent males (41), and premenarcheal girls (42). Recently, Ducher et al. (43) demonstrated that training for more than 12 mo in tennis player females aged 10 to 17 y led to significant gain in bone mass and geometry. The benefits were observed in both pre/perimenarcheal and postmenarcheal females, although the benefits were greater in the former. From these reports and our results, it seems likely that the effect of physical activity on bone growth is greater in the pre/perimenarcheal females than in postmenarcheal females. Although the most effective kind and duration of exercise for bone growth remains unknown, the threshold of habitual mean physical activity intensity per day leading to bone growth was suggested to be around 1.7 METs in Japanese growing males and premenarcheal females.

BMI was a key contributing factor that was significantly associated with BAR in our female participants, though the association was not observed in males. Miyabara et al. (44) conducted a cross-sectional survey and suggested that BMI and physical activity were factors that affected the lumbar bone mineral density of Japanese women aged 19–25 y, with BMI as a key contributing factor. In our participants aged 8–14 y, sex differences were observed in the relation between BMI and BAR. The reason for these sex differences with regard to BMI contribution to bone growth is unclear, but Ito et al. also showed a sex difference with regard to BMI. BMI had a significant association with bone mass in four age groups of Japanese females (9–12, 13–15, 16–18, and 19–22 y), but in males aged 19–22 y, bone mass showed no association with BMI (45). Further study of growing children is required to clarify the reasons. Low BMI is a well-known fracture risk factor. It seems that a higher BMI value has an effect like that of exercise in the point that the mechanical loading stimulates the bone. On the other hand, obesity itself is considered to be a risk factor for fracture in children (46). Obese children are generally sedentary, and consequently they have poor musculoskeletal coordination and their relatively immature skeletons must bear a heavy body weight when they fall, resulting in fractures (3). Therefore, it is necessary to prevent children from being either lean or obese.

It was difficult to use a validated method for defining sexual maturation for Japanese similar to the Tanner stage method for Caucasians (13). Accordingly, the secondary sexual characteristics were assessed by the beginning of menarche in females in our study. In our total population of female participants, the length of time since menarche was also a positive influential factor for BAR after adjusting for age and other confounding factors. Previous reports showed that low age at menarche was associated with the calcaneus growth in Japanese girls aged 10–11 y (13), and forearm bone mineral density in the postmenarcheal group was significantly higher than that in the premenarcheal group among Japanese girls aged 11–13 y (12). Unlike the male and premenarcheal female participants, manageable factors such as nutrient intake and mean physical activity intensity per day were not observed as positive influential factors for BAR in the postmenarcheal females. Henwood and Binkovitz (3) suggested that hormones such as growth hormone and estrogen were essential to normal bone metabolism and mineralization. It has been reported that growth hormone stimulated cell proliferation of trabecular and stromal osteoblasts in vitro (47), and estrogen deficiency led to increased osteoclast formation in the young skeleton (48). These hormones have anabolic effects on bone growth, and pubertal initiation tends to occur earlier in females than males (49). From these reports and our results, it seems that effects of hormones on bone growth may be relatively stronger than those of nutrients and physical activity in postmenarcheal females. It was suggested that dietary intake and physical activity from prepuberty were important to obtain higher maximal bone quantity. Therefore, it is recommended that the intervention to prevent osteoporosis should be started before puberty, at least in girls.

Our study has some limitations. One of them is the cross-sectional design, with which it is difficult to determine causality. A longitudinal study is required to confirm the findings obtained in our study. The second limitation is that bone measurement was done by QUS. Because bone mineral density measured by DXA is recognized as standard for the diagnosis of osteoporosis, QUS parameters (BAR) refer to the percentage of the area that bone trabeculae (ossein) occupy in the sectional area; hence, QUS parameters may not always correspond to bone mass. However, many investigators have
found that QUS parameters are correlated with bone mineral density by DXA, and QUS methods have several advantages, such as no radiation exposure, low cost, and portability. Informed consent for measuring school children by a method that includes radiation exposure is difficult to obtain from parents. Furthermore, QUS parameters reflect the bone stiffness, because these parameters are dependent on the density, the micro- and macrostructure, and the elastic modulus (50). Although QUS parameters may not always correspond to bone mass, these properties are important for predicting bone fracture risk, and QUS methods have been applied to many studies in Japan (13, 19) and abroad (49, 51). Therefore, in our study bone status was evaluated by QUS (Benus III) as an alternative noninvasive method that is suitable for screening the bone status of school-aged children (52). Regarding other limitations, although there was no significant difference in the mean values of BAR between the two adjacent ages among the participants at ages 11 and 14, this may have been due to the small number of participants in these two age groups. Further detailed study is required to determine the differences. It seems that the collection of nutritional and lifestyle information by self-report also has inherent limitations. The validity of some nutrients obtained by BDHQ-10y and BDHQ-15y was confirmed with biomarkers (21), but this was not the case in all of the measured nutrients. Although the evidence is imperfect, our findings nonetheless seem useful for understanding children’s food habits and for making a relative comparison of nutritional intake among the participants.

In conclusion, the results of this study suggest that several manageable factors correlate with bone growth, and the associations differ depending on gender and menarcheal status; a threshold of nutrient intake and physical activity level may exist for bone growth. Our study provided new data on the relationship among age, gender, menarcheal status, a threshold of nutrient intake and physical activity, and the associations differ depending on gender and adolescents. Informed consent for measuring school children and adolescents is required to determine the differences. In our study bone status was evaluated by QUS (Benus III) as an alternative noninvasive method that is suitable for screening the bone status of school-aged children (52). Regarding other limitations, although there was no significant difference in the mean values of BAR between the two adjacent ages among the participants at ages 11 and 14, this may have been due to the small number of participants in these two age groups. Further detailed study is required to determine the differences. It seems that the collection of nutritional and lifestyle information by self-report also has inherent limitations. The validity of some nutrients obtained by BDHQ-10y and BDHQ-15y was confirmed with biomarkers (21), but this was not the case in all of the measured nutrients. Although the evidence is imperfect, our findings nonetheless seem useful for understanding children’s food habits and for making a relative comparison of nutritional intake among the participants.

In conclusion, the results of this study suggest that several manageable factors correlate with bone growth, and the associations differ depending on gender and menarcheal status; a threshold of nutrient intake and physical activity level may exist for bone growth. Our study provided new data on the relationship among age, gender, menarcheal status, nutrients, physical activity, and bone growth by QUS measurements for Japanese growing children.

Acknowledgments
We would like to thank all children, their parents/guardians, and school personnel for participating in this study.

REFERENCES
22) Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O’Brien WL, Bassett DR Jr, Schmitz KH,


41) Viljakainen HT, Natri AM, Kärkkäinen M, Huttenen